Report Title: Test For Chemical Induction of Unscheduled DNA Synthesis In Primary Cultures of Rat Hepatocytes (by Autoradiography)

Test Type: Genotoxicity Study

Conducting Laboratory and Location: Microbiological Associates, Bethesda, MD

Test Substance(s): G0539.01 – Octopirox in ethanol

Species: Rat hepatocytes

Test Conditions: Rat primary hepatocytes injected with 6 doses of test material at levels ranging from 0.2 ug/ml to 50 ug/ml.

Results: Did not cause a significant increase in the mean number of net nuclear grain counts. Test article negative on this study.

Study #: T2982.380
Report Date: 6/28/85
QA statement/GLP compliance: Yes

Accession #: 30980

TEST FOR CHEMICAL INDUCTION OF UNSCHEDULED DNA SYNTHESIS IN PRIMARY CULTURES OF RAT HEPATOCYTES (BY AUTORADIOGRAPHY)

TEST ARTICLE
G0539.01

DRD # BYCR0394

FINAL REPORT

FOR

THE PROCTER & GAMBLE COMPANY
P. O. BOX 39175
CINCINNATI, OHIO 45247

BY

MICROBIOLOGICAL ASSOCIATES, INC.
5221 RIVER ROAD
BETHESDA, MARYLAND 20816

COPY SENT TO: Bettie Evans
Jim Weaver
DATE: 7-8-85

QUALITY ASSURANCE STATEMENT

Study Title: TEST FOR CHEMICAL INDUCTION OF UNSCHEDULED DNA SYNTHESIS IN PRIMARY CULTURES OF RAT HEPATOCYTES (BY AUTORADIOGRAPHY)

Study Number: T2982.380

Study Director: Rodger D. Curren, Ph.D.

Initiation Date: 85/05/07

Review Completed Date: 850628

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the Good Laboratory Practice regulations and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of the study.

INSPECT ON 85/04/29 - 85/04/29,TO STUDY DIR 85/04/29,TO MGMT 85/04/29

PHASES: PROTOCOL REVIEW

INSPECT ON 85/05/21 - 85/05/21,TO STUDY DIR 85/05/21,TO MGMT 85/06/28

PHASES: PREPARATION OF THE HEPATOCYTES

INSPECT ON 85/06/27 - 85/06/27,TO STUDY DIR 85/06/27,TO MGMT 85/06/28

PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joanne K Burke 6/28/85

Quality Assurance
RA/QA Department

Date

TEST FOR CHEMICAL INDUCTION OF UNSCHEDULED DNA SYNTHESIS IN PRIMARY CULTURES OF RAT HEPATOCYTES (BY AUTORADIOGRAPHY)

FINAL REPORT

Test Article : G0539.01

Lot No.: 01

MA Study No.: T2982.380

Divisional Request Document No.: BYCR0394

Test Article Description: White Powder

Storage Conditions: Room Temperature With Desiccation; Protected from Light

Date Received: April 11, 1985

Initiation Date: May 7, 1985

Completion Date: June 28, 1985

Report Date: June 28, 1985

Sponsor: THE PROCTER & GAMBLE COMPANY
P. O. Box 39175
Cincinnati, Ohio 45247

Sponsor's Investigator: J. E. Weaver

Testing Facility: MICROBIOLOGICAL ASSOCIATES, INC.
5221 River Road
Bethesda, Maryland 20816

Study Director: Rodger D. Curren 6/28/85
Rodger D. Curren, Ph.D. Date

Laboratory Supervisor: Margaret Kung 6/28/85
Margaret Kung, B.S. Date

Laboratory Technician: Linda L. Dunn 6/28/85
Linda Dunn, B.S. Date

SUMMARY

Procter & Gamble's test article, G0539.01, was tested in the Unscheduled DNA Synthesis Test using rat primary hepatocytes. Based on the results of an initial toxicity test, the test article was tested at 6 dose levels ranging from 0.2 ug/ml to 50 ug/ml.

The results of the UDS assay indicate that under the test conditions, the test article did not cause a significant increase in the mean number of net nuclear grain counts (i.e., an increase of at least 5 counts over the control), at any dose level. Therefore, the test article is considered negative in this study.

INTRODUCTION

This study was conducted by R. Curren, Ph.D., Margaret Kung, B.S. and Linda Dunn, B.S. from 5/7/85 to 6/25/85 at Microbiological Associates. The experimental procedure employed was essentially that described by Williams, G.M. (Cancer Research 37:1845-1851, 1977) and is described in detail in the appendix to this report.

The purpose of the study was to evaluate the test article, G0539.01, for its ability to induce Unscheduled DNA Synthesis in rat primary hepatocytes as measured by autoradiographic methods.

MATERIALS AND METHODS

Indicator Cells

Primary rat liver cell cultures derived from the livers of normal adult male Sprague-Dawley rats were used in this study. The animals were obtained from the Frederick Cancer Research Facility and were quarantined for at least one week prior to the initiation of the study. The animals were maintained on standard laboratory diet throughout the quarantine period.

The procedure used for obtaining rat hepatocyte cultures (HPC) was essentially that of Williams, et al., (In Vitro 13:809-817, 1977). Each rat used was sacrificed by inhalation of metofane. The animal was dissected and perfused first with 0.5mM EGTA solution and then with a collagenase solution. The liver was removed from the animal and the cells were dissociated, counted, and seeded into 35 mm dishes containing coverslips (5.0×10^5 viable cells/dish). The cells were seeded in Williams Medium E (WME) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units of penicillin and 100 ug of streptomycin/ml. The cultures were incubated at 37 $\pm$ 1°C in a humidified 5% CO_2 incubator for 90-120 minutes, washed and refed with serum-free medium and used in the test.

Test and Control Articles

The test article, G0569.01, was received on 4/11/85, and stored at room temperature in the dark. The test article was dissolved and diluted in Ethanol (Pharmco, Designated Lot #1-11-85) to make up the stock solutions. 7,12-Dimethylbenzanthracene (DMBA), Kodak Lot C9C, was dissolved in DMSO, Aldrich Lot #9727 CL), and used as a positive control in this study.

The test article was diluted to appropriate concentrations immediately prior to use. Approximately 20 to 30 minutes elapsed between the time the test article was dissolved and the final treatment of cells. All test article and control treatments were done under subdued yellow lights to avoid possible problems of photoinactivation.

Documentation of the derivation, characterization, and stability testing of the test substance will be the responsibility of the Sponsor.

Identification of Test System

All culture plates were labeled with an indelible pen with a code system which clearly identifies the test article or control, test phase, and the experiment number. Slides were similarly labeled with pencil.

Initial Cytotoxicity Test

A preliminary cytotoxicity test was performed to establish an appropriate dose range for the test article. Ten doses ranging from 0.02 ug/ml to 800 ug/ml were tested. The test article was tested by treating replicate cultures of HPC 90-120 minutes after seeding. Eighteen hours later, the cells were washed with Ca^{++}, Mg^{++} free phosphate buffered saline (PBS), trypsinized, exposed to trypan blue and counted in a hematocytometer. Two replicate plates were used for counting at each dose level. The relative survival indices (RSI) were obtained by comparing the treated to control groups.

Unscheduled DNA Synthesis Test

Based on the results of the initial cytotoxicity test, the test article, G0539.01, was tested at 6 dose levels. Three replicate plates seeded with 5.0×10^5 HPC/plate were treated with 0.2 ug/ml to 50 ug/ml of test article. DMBA, at 3 ug/ml and 10 ug/ml, was used as the positive control. Ethanol, which was used to dissolve G0539.01, was used as the solvent control for the test article. DMSO, which was used to dissolve the positive control was used as the solvent control for DMBA. Each test article and control dish received ^3H-thymidine at a final concentration of 10 uCi/ml. In parallel with the test plates, three cultures per dilution were treated with the same compound for a parallel toxicity test.

The cells were treated for 18 hours as described earlier. The parallel toxicity plates were harvested by trypsinization and viable cell counts were made as described in the initial cytotoxicity test to obtain the relative survivals and relative toxicities.

After eighteen hours of exposure, the cells in the Unscheduled DNA Synthesis assay plates were washed in serum-free WME, swelled in 1% sodium citrate and fixed in ethanol-acetic acid fixative. The coverslips were air-dried, mounted cell side up on glass slides, and allowed to dry. The slides were coated with Kodak NTB emulsion and stored for 10 days at 4°C in light tight boxes with desiccant. The slides were then developed in Kodak D-19 developer, fixed in Kodak fixer and stained in hemotoxylin-sodium acetate-eosin stain.

Scoring

The slides were read "blind" on an Artek Colony Counter. Nuclear grains were counted in 25 cells in random areas on each of 2 coverslips per treatment. The net nuclear counts were determined by counting three nucleus-sized areas adjacent to each nucleus and subtracting the average cytoplasmic count from the nuclear count. Replicative synthesis was identified by nuclei completely blackened with grains and such cells were not counted. Nuclei exhibiting toxic effects of treatment, such as dark staining disrupted membranes or irregular shape, were not counted. Nuclei with a projected image of less than 4.0 mm^2 were also not counted.

Analysis of Data

For each treatment slide and for each dose level, the net nuclear counts were averaged and a standard deviation (S.D.) was determined by using an IBM XT with a LOTUS 1-2-3 program. These values along with the Grand Mean (mean of net nuclear counts from all 50 cells at each dose level) and standard deviation and the percent of cells at each dose level having $\geq$ 5 net nuclear counts are reported on a summary form.

Criteria for Evaluation of Test Results

The results of this study were evaluated according to the criteria described below.

If the mean net nuclear count is increased by at least five counts over the control, the results for a particular dose level will be considered significant. A test article will be judged positive if it induces a dose-related response and at least one dose produces a significant increase in the average net nuclear grains when compared to that of the control. In the absence of the dose response, the test article should show a significant increase in the mean net nuclear grain count in at least two successive doses. If a test article showed a significant increase in the net nuclear grain count at one dose level without any dose response, the test article will be considered to have a marginal positive activity. The test article will be considered negative if no significant increase in the net nuclear grain counts at any dose level is observed.

Records

All raw data, final report and stained slides of this study are maintained in the archives of Microbiological Associates, Inc. located at 5221 River Road, Bethesda, Maryland 20816.

RESULTS AND DISCUSSION

In the initial cytotoxicity assay, the test article, G0539.01, showed a relative toxicity (RT) of 58.6% at 80 ug/ml and a RT of -36.0% at 8 ug/ml (Table 1). Therefore, the UDS assay was conducted using 6 decreasing test article concentrations ranging from 50 ug/ml to 0.2 ug/ml.

The results of the parallel cytotoxicity assay are recorded in Table 2. The maximum dose of test article (50 ug/ml) caused an RT of 54.3% and the next lower dose of 17 ug/ml showed an RT of 47.4%. The lowest dose of 0.2 ug/ml showed a RT of -1.4%.

The results of the UDS assay are summarized in Table 3. Each slide treated with G0539.01 or DMBA was compared to the appropriate negative control. According to the criteria set for evaluating the test results, both doses of the positive control compound, DMBA, induced a significant increase in the average net nuclear count of silver grains. None of the test article doses caused a significant increase in the mean net nuclear counts.

Study No. T2982.380

TABLE 1

INITIAL TOXICITY TEST

UNSCHEDULED DNA SYNTHESIS

TREATMENT	DISHES COUNTED	% VIABLE CELLS	VIABLE CELLS/DISH (X10^5)	SURVIVAL INDEX	RELATIVE SURVIVAL	RELATIVE TOXICITY
G0539.01						
800 ug/ml	2	4.1%	0.090	1.8%	5.4%	94.6%
200 ug/ml	2	35.0%	0.780	15.6%	46.8%	53.2%
80 ug/ml	2	43.7%	0.690	13.8%	41.4%	58.6%
20 ug/ml	2	56.2%	0.780	15.6%	46.8%	53.2%
8 ug/ml	2	67.7%	2.265	45.3%	136.0%	-36.0%
2 ug/ml	2	66.4%	1.545	30.9%	92.8%	7.2%
0.8 ug/ml	2	69.2%	1.470	29.4%	88.3%	11.7%
0.2 ug/ml	2	69.9%	1.560	31.2%	93.7%	6.3%
0.08 ug/ml	2	68.0%	1.875	37.5%	112.6%	-12.6%
0.02 ug/ml	2	67.5%	2.070	41.4%	124.3%	-24.3%
ETHANOL						
10 ul/ml	2	65.5%	1.665	33.3%	100.0%	0.0%
WME	2	78.0%	2.580	51.6%	155.0%	-55.0%

Cells plated per dish: 500,000

$$\text{Survival Index} = \frac{\text{Average Viable Cells per Dish} \times 100}{\text{Cells Plated per Dish}}$$

$$\text{Relative Survival} = \frac{\text{Survival Index} \times 100}{\text{Survival Index of Control}}$$

Relative Toxicity = 100% - Relative Survival

WME = Untreated Control

TABLE 2

PARALLEL TOXICITY TEST

UNSCHEDULED DNA SYNTHESIS

TREATMENT	DISHES COUNTED	% VIABLE CELLS	VIABLE CELLS/DISH (X10^5)	SURVIVAL INDEX	RELATIVE SURVIVAL	RELATIVE TOXICITY
G0539.01						
50 ug/ml	3	53.1%	1.340	26.8%	45.7%	54.3%
17 ug/ml	3	61.2%	1.540	30.8%	52.6%	47.4%
5.0 ug/ml	3	76.1%	2.630	52.6%	89.8%	10.2%
1.7 ug/ml	3	76.3%	2.340	46.8%	79.9%	20.1%
0.5 ug/ml	3	74.8%	2.570	51.4%	87.7%	12.3%
0.2 ug/ml	3	77.8%	2.970	59.4%	101.4%	-1.4%
DMBA						
10 ug/ml	3	68.4%	1.150	23.0%	42.4%	57.6%
3 ug/ml	3	72.9%	2.070	41.4%	76.4%	23.6%
DMSO (Solvent Control For DMBA)						
10 ul/ml	3	84.4%	2.710	54.2%	100.0%	0.0%
ETHANOL (Solvent Control For Test Article)						
10 ul/ml	3	80.8%	2.930	58.6%	100.0%	0.0%
WME	3	79.7%	3.280	65.6%	111.9%	-11.9%

Cells plated per dish: 500,000

$$\text{Survival Index} = \frac{\text{Average Viable Cells per Dish} \times 100}{\text{Cells Plated per Dish}}$$

$$\text{Relative Survival} = \frac{\text{Survival Index} \times 100}{\text{Survival Index of Control}}$$

Relative Toxicity = 100% - Relative Survival

WME = Untreated Control

Study No. T2982.380

TABLE 3
SUMMARY OF UDS ASSAY
WITH TEST ARTICLE G0539.01

TREATMENT	RELATIVE SURVIVAL	SLIDE DESIGNATION	NO. OF NUCLEI COUNTED	AVERAGE NET GRAINS PER NUCLEUS		S.D.	GRAND MEAN		S.D.	PERCENT CELLS WITH 5 OR MORE NET NUCLEAR GRAINS
G0539.01										
50 ug/ml	45.7%	40A	25	0.2	+/-	0.9	0.4	+/-	1.0	0.0%
		40B	25	0.6	+/-	1.1				
17 ug/ml	52.6%	42A	25	-0.3	+/-	0.7	0.0	+/-	0.9	0.0%
		42B	25	0.2	+/-	0.9				
5.0 ug/ml	89.8%	44A	25	0.0	+/-	0.8	0.0	+/-	0.9	0.0%
		44B	25	0.1	+/-	0.9				
1.7 ug/ml	79.9%	41A	25	0.2	+/-	0.5	0.2	+/-	0.7	0.0%
		41B	25	0.2	+/-	0.9				
0.5 ug/ml	87.7%	45A	25	0.1	+/-	0.8	0.2	+/-	0.7	0.0%
		45B	25	0.3	+/-	0.7				
0.2 ug/ml	101.4%	47A	25	0.0	+/-	0.6	0.0	+/-	0.7	0.0%
		47B	25	0.0	+/-	0.8				
DMBA										
10 ug/ml	42.4%	24A	25	17.7	+/-	3.7	17.5	+/-	4.2	100.0%
		24B	25	17.3	+/-	4.7				
3 ug/ml	76.4%	29A	25	13.4	+/-	2.8	13.1	+/-	2.9	100.0%
		29B	25	12.7	+/-	3.1				
DMSO (Solvent Control For DMBA)										
10 ul/ml	100.0%	26A	25	-0.3	+/-	0.6	-0.1	+/-	0.6	0.0%
		26C	25	0.1	+/-	0.6				
ETHANOL (Solvent Control for Test Article)										
10 ul/ml	100.0%	46A	25	0.2	+/-	0.5	0.2	+/-	0.6	0.0%
		46B	25	0.1	+/-	0.7				
WME	111.9%	23A	25	0.1	+/-	0.7	0.0	+/-	0.7	0.0%
		23B	25	-0.1	+/-	0.7				

WME = Untreated Control
S.D. = Standard Deviation

CONCLUSION

Procter & Gamble's test article, G0539.01, was tested in the Rat Hepatocyte Unscheduled DNA Synthesis Assay. Based on the results of the initial toxicity test, the test article was tested at 6 dose levels ranging from 0.2 ug/ml to 50 ug/ml.

The results of the UDS assay indicate that under the test conditions, the test article did not cause a significant increase in the Unscheduled DNA Synthesis as measured by the mean number of net nuclear grain counts (i.e., an increase of at least 5 counts over the control), at any dose level. In this study, the positive control, DMBA, induced significant increases in the mean number of net nuclear grain counts over that in the solvent control. All criteria for a valid test were met.

APPENDIX

THE PROCTER & GAMBLE COMPANY

...NED BY RAQA 4/16/85

PROTOCOL C30?

Test for Chemical Induction of Unscheduled DNA Synthesis in Primary Cultures of Rat Hepatocytes (By Autoradiography)

Issue Date: December 14, 1982

Supersedes Issue Dated: June 20, 1982

Test Substance Identification Number (TSIN) # G0539.01

Divisional Request Document Number (DRD) # BYCR0394

Sponsor: The Procter & Gamble Company
Cincinnati, Ohio

Testing Facility: (To be filled in by Operations Section)
Microbiological Associates, Inc.
5221 River Road
Bethesda, MD 20016

Study # T2982380 (To be filled in by Testing Facility)

Purpose: To determine whether a test substance elicits Unscheduled DNA Synthesis (UDS) in primary cultures of rat hepatocytes. (IN VITRO)

Justification for Selection of Test System: Primary rat hepatocytes serve as the system of choice due to the amount of background data available, and their endogenous metabolic activation capacity.

Route of Administration of Test Substance and Reason for Choice: IN VITRO. Route specified by test procedure.

Records to be Maintained: Preparation of primary rat hepatocyte cultures. Documentation of test substance preparation, preparation of cells, dosing, and grain counts. Include any other records that would be required to reconstruct the study and demonstrate adherence to protocol.

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Test Substance(s)

TSIN #	DRD Number	Description: Color	Description: Physical Form	Expiration Date
G0539.01	BYCR0394	White	Powder	12/7/85

Storage Conditions: (Check one)

[ ] Room temperature [ ] Refrigerator [ ] Freezer
[XX] Other Ambient (50°-90°F)

Hazards: (Check one)

[ ] None known. Take ordinary precautions in handling.
[XX] As follows: Irritation JEW 3/13/85

Special Instructions: (Check one)

[ ] None
[XX] As follows: Aoivd contact with eye and undue skin contact. Flush with water.

Dose Preparation:

Vehicles in order of preference

[1] Water
[ ] DMSO
[2] EtOH
[3] Acetone
[ ] Other

Solubility Water = ~~1%~~ < 1%. Ethanol & Acetone ~~5%~~ > 10% NAD 4/2/85

Unless the solubility properties of the test substance are provided by the Sponsor or the solubility properties are available from another source, a suitable solvent must be found for the test substance prior to testing using the Standard Operating Procedures of the Test Facility.

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Chemicals:

Positive controls and other chemicals to be used for testing will be purchased from a commercial source or obtained from the Sponsor. Chemicals are stored according to the recommendations of the commercial supplier or Sponsor. After completion of the assay, unused commercially obtained chemicals may be saved for future use. Excess chemicals obtained from a Sponsor, however, will be either returned or discarded at the discretion of the Sponsor.

Preparation of Dosing Solutions:

Using the Standard Operating Procedures of the Test Facility, immediately prior to each assay, test articles will be diluted in the appropriate solvent to form a series of concentrations that when diluted into culture medium will yield the appropriate set of test concentrations. The final concentration of solvent will be maintained at 1% or less to minimize the possibility of a cytotoxic effect in response to the solvent.

Both solvent and positive controls will be used in every UDS assay. The positive control will be 7,12-dimethylbenzanthracene, a chemical known to induce UDS in this system (1).

Note

A concentration analysis of the test substance - vehicle mixture(s) will [X]; will not [ ] be required.

If a concentration analysis is required:

[X] Prepare a sufficient quantity of the most concentrated test substance - vehicle mixture(s) so that a portion can be returned to the Sponsor's Divisional Toxicologist.

Shipping Instructions

Send approximately 50-100 ml. Send [ ] frozen; [X] under ambient conditions; [ ] other_____

[ ] Analyze the test substance - vehicle mixture(s) for test substance concentration using the analytical method in Appendix _____.

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Test System Identification: Individual cultures are to be identified according to the Standard Operating Procedures of the Test Facility.

Test System: Primary cultures of Fisher 344 or Sprague-Dawley rat hepatocytes prepared by in situ perfusion with collagenase according to the method of Williams et al (2).

Methods:

Primary Cell Culture

Primary rat hepatocytes isolated by in situ collagenase perfusion of adult, male Fisher 344 or Sprague-Dawley rats will be used for the UDS assay. The Standard Operating Procedures of the Test Facility for culture preparation are based on the procedure of Williams et al (1, 2). Cultures will be initiated on coverslips in a suitable tissue culture vessel in Williams Medium E supplemented with 10% (v/v) fetal bovine serum and antibiotics. After a period of 1.5-2 hours at 37 ± 1.0°C, the attached cells will be washed to remove floating (nonviable) cells. Cells will then be refed and incubated at 37 ± 1.0°C in an atmosphere of 5% CO_2 in air unless used immediately for a UDS or toxicity assay. Because the ability of the hepatocytes to metabolically activate promutagens drops quickly in culture, only cultures freshly prepared on the same day will be used for the UDS assay.

Preliminary Toxicity Test

A preliminary cytotoxicity test will be performed according to the Standard Operating Procedures of the Test Facility to establish the appropriate dose range for the UDS assay. The index of viability that will be used following exposure to test chemical will be exclusion of trypan blue (0.04-0.08%). The maximum dose chosen for the UDS assay, if possible, will be one that induces at least a 50% reduction in cell viability relative to the solvent control. Subsequent doses will

Methods (Cont'd): Preliminary Toxicity Test (Cont'd)

be chosen to span a range down to no apparent relative toxic effect. If no relative toxic effect is observed at any dose, the doses chosen for the UDS assay will be based on the solubility of the test substance. In this case, the highest dose tested should exceed the solubility of the test substance or be ~~100~~ 10 mg/ml, whichever is smaller. Test liquids may be tested on the basis of volume with ~~100~~ 10 µl/ml being the highest dose tested in the absence of toxicity. NLD 4/2/85

UDS Test

For each UDS assay, three or four freshly prepared hepatocyte cultures will be used for each dose of the test substance. At least five doses chosen on the basis of a preliminary toxicity assay or a previous UDS assay will be used. Using the Standard Operating Procedures of the Test Facility, cultures will be exposed to both test substance and 10 µCi/ml ^{3}H-thymidine (specific activity 20-60 Ci/mmole) for 18-20 hours at 37 ± 1.0°C under an atmosphere of 5% CO_2 in air. Exposures will be done in serum-free Williams Medium E.

Following the exposure period, the cultures will be scored for toxicity or washed with a buffered, balanced salt solution and then processed for autoradiography according to the Standard Operating Procedures of the Test Facility.

Incorporation of ^{3}H-thymidine into nuclear DNA will then be determined by counting darkened grains localized over nuclei in at least 50 randomly chosen but normal appearing cells per dose group. (Cells visibly suffering from toxic effects of treatment will not be scored, i.e. constricted cells, irregularly shaped, very darkly stained, etc.) The 50 cells will be chosen from at least two coverslips per dose group. The counts for both nuclei of binucleated cells will be recorded separately. Background incorporation will be determined by counting at least two nucleus-sized areas of cytoplasm adjacent to each nucleus. Net nuclear grain counts will be determined by subtracting the appropriate background. All grain counts will be done using an electronic colony counter (such as an

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
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Issue Date: December 14, 1982

Methods (Cont'd): Artek 880 or 980) equipped with a microscope-mounted auxiliary T.V. camera. Grain counts may be done either directly by using the "count" mode of the colony counter or indirectly by determining the relative area covered by darkened grains by using the "area" mode of the counter. Area counts may then, in turn, be related to minimum numbers of grains by comparison to the average grain size. Data for each coverslip scored will be reported separately.

Protocol Changes: If it becomes necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and the Sponsor. As soon as practical, this change and the reasons for it should be put in writing and signed by both the Study Director and the Sponsor's Divisional Toxicologist. This document is then attached to the protocol as an addendum.

Results: The raw data are recorded for each negative and positive control and each dose of test substance. Raw data consist of individual grain counts and dose preparation information.

The mean net grain count ± the standard deviation or error is reported for each coverslip culture scored.

Because the primary hepatocyte cultures employed in this assay may be composed of heterogeneous populations of cells with varying metabolic and repair capacities, the observed net nuclear grain counts may be a complex distribution. As a result, before reaching conclusions based on the data, statistical analysis employing "t" or ANOVA tests may be applied to the mean or median grain counts for each coverslip. Such analysis will be done at the direction of the Sponsor or by the Sponsor or his/her representative.

Results of each test will be considered independently, but in order to be considered a valid test, solvent and positive control mean net nuclear grain counts should fall in a proper historical range. Repeat testing may sometimes be required in some cases such as sporadic or apparent single dose responses.

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Report:

Final Report

A report of the results will be prepared for this study by the contract facility. The report will include, but not be limited to, the following:

1. Name and address of the facility performing the study and the dates on which the study was initiated and completed.

2. Objectives as stated in the approved protocol, and any changes to the original protocol.

3. A detailed description of all methods used.

4. Statistical methods employed for analysis of the data, if any.

5. Deviations from the Test Facility's Standard Operating Procedures or the approved protocol.

6. A summary of the results as they relate to the study's objective.

7. The location where all raw data will be stored.

This report shall conform to all requirements outlined in Section 58.185, Subpart J, Good Laboratory Practices Regulations.

cc: Client copy

THE PROCTER & GAMBLE COMPANY

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Sponsor: James E. Weaver James E Weaver
Divisional Toxicologist

Date Approved by Sponsor's Divisional Toxicologist 3-7-85

Proposed Starting Date: May 1, 1985
Defined as Preliminary Cytotoxicity

Proposed Completion Date: 6/19/85
Defined as Final Report Completion

(To be completed by the Test Facility)

Study Director: Rodger D. Curtis

Date: April 16, 1985

Study Cost: $5,200.00

References:

1. G. M. Williams. The detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. Cancer Res. 37: 1845-1851 (1977).

2. G. M. Williams, E. Bermudez, and D. Scaramuzzino. Rat hepatocyte primary cell cultures. III. Improved dissociation and attachment techniques and the enhancement of survival by culture medium. In Vitro 13: 809-817 (1977).

MICROBIOLOGICAL ASSOCIATES INC.

Microbiological Associates Inc.
5221 River Road
Bethesda, Maryland 20816
(301) 654-3400
Telex: 90-8793

A Subsidiary of
Daryl Laboratories Inc

June 28, 1985

Mr. Hal A. Derner
Procter & Gamble Company
P.O. Box 39175
Cincinnati, Ohio 45247

Dear Mr. Derner:

Enclosed please find 4 copies of the Final Report for Test Article G0539.01, Lot No. 01 (MA Study No. T2982.380.

If you have any questions please do not hesitate to contact us.

Sincerely,

Cheryl Respess

Cheryl Respess
Genetic Toxicology

cc: J. Stinnett
R. Curren

Enclosure

MICROBIOLOGICAL ASSOCIATES INC.

Microbiological Associates Inc.
5221 River Road
Bethesda, Maryland 20816
(301) 654-3400
Telex: 90-8793

A Subsidiary of
Daryl Laboratories Inc

April 16, 1985

Mr. H.A. Derner
The Procter & Gamble Company
Miami Valley Laboratories
P.O. Box 39175
Cincinnati, Ohio 45247

Dear Mr. Derner:

Enclosed is a completed and signed copy of your Protocol No. C30B for an Unscheduled DNA Synthesis Assay on your test article G0539.01, (BYCR0394), (MA Study No. T2982.380).

The price for this study will be:

Unscheduled DNA Synthesis Assay (Includes 2 Copies of Final Report)	$5,200
Extra Copies of Final Report (2 Copies @ $5)	10
Shipping and Handling (Return Test Article U.P.S. - Room Temp.)	20
	$5,230

The cost of returning the test article for both the UDS (.380) study and the Mouse Lymphoma study (.701) being performed on this test article is included as a one-time charge to the UDS study, as referenced above.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Janet C. Stinnett
Admin. Asst.
Division of Genetic Toxicology

/jcs

Enclosure

THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O BOX 39175
CINCINNATI, OHIO 45247

April 11, 1985

Dr. Steve Haworth
Microbiological Associates, Inc.
5221 River Road
Bethesda, MD 20016

Dear Dr. Haworth:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Notice: 1) This study is expected to be submitted to the following regulatory agency: FDA. The study should be listed on the Test Facility's Master list of regulated studies. The stipulations of the protocol are to be implemented in complete conformance with Good Laboratory Practices Regulations (21 CFR, Part 58) for nonclinical laboratory studies with the following exceptions:

If two or more test substances appear on the protocol, it may be conducted as a single study, resulting in a single final report.

2) Quality Assurance Inspections:

The final report will be inspected by the Test Facility's QAU. The Test Facility's Standard Operating Procedure for randomly inspecting all operations should be used to assure study validity and sufficient data should be made a part of each report to allow the Sponsor to check the reported results against the raw data.

3) Documentation of the derivation, characterization, and stability testing of the test substance(s) will be the responsibility of the Sponsor.

Test: Unscheduled DNA and Synthesis
Protocol No.: C30B Issue Date: December 14, 1982
Test Substance No.: G0539.01 Doc. Req. No.: BYCR 0394
Physical Form: Powder

Three copies of the final report are needed as soon as possible, and are to be sent to my attention at the above address.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Divisional Toxicologist or Ms. J. A. Skare. All unused samples are to be returned to the Divisional Toxicologist at the following address (the cost of shipment should be included in the study cost):

Mr. J. E. Weaver Telephone No. (513) 530-2302
The Procter & Gamble Company
Sharon Woods Technical Center
11511 Reed Hartman Hwy. - Room HB-2D31
Cincinnati, OH 45241

Complete both copies of the attached protocol by adding your study number, proposed start and completion dates, and have the Study Director sign and date them. The Study Director should define the start and completion dates on the protocol. Retain one copy and return one copy (which includes the study cost) to me along with a letter stating that you agree to do the work specified in the attached protocol. In addition, if you cannot meet the report dates, please let me know.

An invoice for 80% of the amount should be sent to:

Mr. R. T. Lyons
The Procter & Gamble Company
11511 Reed Hartman Highway - Room No. HB-2D31
Cincinnati, OH 45241

An invoice for 20% of the amount should be sent to:

Mr. Detlef Müller
The Procter & Gamble Company
European Technical Center
Temselaan 100, B 1820-Grimbergen
(Strombeek-Bever)
BELGIUM

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research & Development Department

H. A. Derner
Human & Environmental Safety Division

Approved: _____
G. S. Hassing, Ph.D
Director, Human & Environmental Safety Division

bg
Attachments
cc: Study File
J. E. Weaver
J. A. Skare
D. Müller

Client copy

THE PROCTER & GAMBLE COMPANY

PROTOCOL C30?

Test for Chemical Induction of Unscheduled DNA Synthesis in Primary Cultures of Rat Hepatocytes (By Autoradiography)

Issue Date: December 14, 1982

Supersedes Issue Dated: June 20, 1982

Test Substance Identification Number (TSIN) # G0539.01

Divisional Request Document Number (DRD) # BYCR0394

Sponsor:	The Procter & Gamble Company Cincinnati, Ohio	
Testing Facility: (To be filled in by Operations Section)	Microbiological Associates, Inc. 5221 River Road Bethesda, MD 20016	Study # T2982.380 (To be filled in by Testing Facility)
Purpose:	To determine whether a test substance elicits Unscheduled DNA Synthesis (UDS) in primary cultures of rat hepatocytes. (IN VITRO)	
Justification for Selection of Test System:	Primary rat hepatocytes serve as the system of choice due to the amount of background data available, and their endogenous metabolic activation capacity.	
Route of Administration of Test Substance and Reason for Choice:	IN VITRO. Route specified by test procedure.	
Records to be Maintained:	Preparation of primary rat hepatocyte cultures. Documentation of test substance preparation, preparation of cells, dosing, and grain counts. Include any other records that would be required to reconstruct the study and demonstrate adherence to protocol.	

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of Unscheduled DNA Synthesis in Primary Cultures of Rat Hepatocytes (By Autoradiography)

Issue Date: December 14, 1982

Test Substance(s)

TSIN #	DRD Number	Description: Color	Description: Physical Form	Expiration Date
G0539.01	BYCR0394	White	Powder	12/7/85

Storage Conditions: (Check one)

[ ] Room temperature [ ] Refrigerator [ ] Freezer
[XX] Other Ambient (50°-90°F)

Hazards: (Check one)

[ ] None known. Take ordinary precautions in handling.
[XX] As follows: Irritation JCW 3/13/85

Special Instructions: (Check one)

[ ] None
[XX] As follows: Aoivd contact with eye and undue skin contact. Flush with water.

Dose Preparation:

Vehicles in order of preference

[1] Water
[ ] DMSO
[2] EtOH
[3] Acetone
[ ] Other

Solubility Water = ~~1%~~ < 1%, Ethanol & Acetone ~~5%~~ > 10% NAD 4/2/85

Unless the solubility properties of the test substance are provided by the Sponsor or the solubility properties are available from another source, a suitable solvent must be found for the test substance prior to testing using the Standard Operating Procedures of the Test Facility.

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Chemicals:

Positive controls and other chemicals to be used for testing will be purchased from a commercial source or obtained from the Sponsor. Chemicals are stored according to the recommendations of the commercial supplier or Sponsor. After completion of the assay, unused commercially obtained chemicals may be saved for future use. Excess chemicals obtained from a Sponsor, however, will be either returned or discarded at the discretion of the Sponsor.

Preparation of Dosing Solutions:

Using the Standard Operating Procedures of the Test Facility, immediately prior to each assay, test articles will be diluted in the appropriate solvent to form a series of concentrations that when diluted into culture medium will yield the appropriate set of test concentrations. The final concentration of solvent will be maintained at 1% or less to minimize the possibility of a cytotoxic effect in response to the solvent.

Both solvent and positive controls will be used in every UDS assay. The positive control will be 7,12-dimethylbenzanthracene, a chemical known to induce UDS in this system (1).

Note

A concentration analysis of the test substance - vehicle mixture(s) will [X]; will not [ ] be required.

If a concentration analysis is required:

[X] Prepare a sufficient quantity of the most concentrated test substance - vehicle mixture(s) so that a portion can be returned to the Sponsor's Divisional Toxicologist.

Shipping Instructions

Send approximately 50-100 ml. Send [ ] frozen; [X] under ambient conditions; [ ] other_____

[ ] Analyze the test substance - vehicle mixture(s) for test substance concentration using the analytical method in Appendix _____.

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Test System Identification: Individual cultures are to be identified according to the Standard Operating Procedures of the Test Facility.

Test System: Primary cultures of Fisher 344 or Sprague-Dawley rat hepatocytes prepared by *in situ* perfusion with collagenase according to the method of Williams et al (2).

Methods:

Primary Cell Culture

Primary rat hepatocytes isolated by *in situ* collagenase perfusion of adult, male Fisher 344 or Sprague-Dawley rats will be used for the UDS assay. The Standard Operating Procedures of the Test Facility for culture preparation are based on the procedure of Williams et al (1, 2). Cultures will be initiated on coverslips in a suitable tissue culture vessel in Williams Medium E supplemented with 10% (v/v) fetal bovine serum and antibiotics. After a period of 1.5-2 hours at 37 ± 1.0°C, the attached cells will be washed to remove floating (nonviable) cells. Cells will then be refed and incubated at 37 ± 1.0°C in an atmosphere of 5% CO_2 in air unless used immediately for a UDS or toxicity assay. Because the ability of the hepatocytes to metabolically activate promutagens drops quickly in culture, only cultures freshly prepared on the same day will be used for the UDS assay.

Preliminary Toxicity Test

A preliminary cytotoxicity test will be performed according to the Standard Operating Procedures of the Test Facility to establish the appropriate dose range for the UDS assay. The index of viability that will be used following exposure to test chemical will be exclusion of trypan blue (0.04-0.08%). The maximum dose chosen for the UDS assay, if possible, will be one that induces at least a 50% reduction in cell viability relative to the solvent control. Subsequent doses will

Methods (Cont'd): Preliminary Toxicity Test (Cont'd)

be chosen to span a range down to no apparent relative toxic effect. If no relative toxic effect is observed at any dose, the doses chosen for the UDS assay will be based on the solubility of the test substance. In this case, the highest dose tested should exceed the solubility of the test substance or be ~~100~~ 10 mg/ml, whichever is smaller. Test liquids may be tested on the basis of volume with ~~100~~ 10 µl/ml being the highest dose tested in the absence of toxicity. NLD 4/2/85

UDS Test

For each UDS assay, three or four freshly prepared hepatocyte cultures will be used for each dose of the test substance. At least five doses chosen on the basis of a preliminary toxicity assay or a previous UDS assay will be used. Using the Standard Operating Procedures of the Test Facility, cultures will be exposed to both test substance and 10 µCi/ml ^{3}H-thymidine (specific activity 20-60 Ci/mmole) for 18-20 hours at 37 ± 1.0°C under an atmosphere of 5% CO_2 in air. Exposures will be done in serum-free Williams Medium E.

Following the exposure period, the cultures will be scored for toxicity or washed with a buffered, balanced salt solution and then processed for autoradiography according to the Standard Operating Procedures of the Test Facility.

Incorporation of ^{3}H-thymidine into nuclear DNA will then be determined by counting darkened grains localized over nuclei in at least 50 randomly chosen but normal appearing cells per dose group. (Cells visibly suffering from toxic effects of treatment will not be scored, i.e. constricted cells, irregularly shaped, very darkly stained, etc.) The 50 cells will be chosen from at least two coverslips per dose group. The counts for both nuclei of binucleated cells will be recorded separately. Background incorporation will be determined by counting at least two nucleus-sized areas of cytoplasm adjacent to each nucleus. Net nuclear grain counts will be determined by subtracting the appropriate background. All grain counts will be done using an electronic colony counter (such as an

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Methods (Cont'd): Artek 880 or 980) equipped with a microscope-mounted auxiliary T.V. camera. Grain counts may be done either directly by using the "count" mode of the colony counter or indirectly by determining the relative area covered by darkened grains by using the "area" mode of the counter. Area counts may then, in turn, be related to minimum numbers of grains by comparison to the average grain size. Data for each coverslip scored will be reported separately.

Protocol Changes: If it becomes necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and the Sponsor. As soon as practical, this change and the reasons for it should be put in writing and signed by both the Study Director and the Sponsor's Divisional Toxicologist. This document is then attached to the protocol as an addendum.

Results: The raw data are recorded for each negative and positive control and each dose of test substance. Raw data consist of individual grain counts and dose preparation information.

The mean net grain count ± the standard deviation or error is reported for each coverslip culture scored.

Because the primary hepatocyte cultures employed in this assay may be composed of heterogeneous populations of cells with varying metabolic and repair capacities, the observed net nuclear grain counts may be a complex distribution. As a result, before reaching conclusions based on the data, statistical analysis employing "t" or ANOVA tests may be applied to the mean or median grain counts for each coverslip. Such analysis will be done at the direction of the Sponsor or by the Sponsor or his/her representative.

Results of each test will be considered independently, but in order to be considered a valid test, solvent and positive control mean net nuclear grain counts should fall in a proper historical range. Repeat testing may sometimes be required in some cases such as sporadic or apparent single dose responses.

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Report:

Final Report

A report of the results will be prepared for this study by the contract facility. The report will include, but not be limited to, the following:

1. Name and address of the facility performing the study and the dates on which the study was initiated and completed.

2. Objectives as stated in the approved protocol, and any changes to the original protocol.

3. A detailed description of all methods used.

4. Statistical methods employed for analysis of the data, if any.

5. Deviations from the Test Facility's Standard Operating Procedures or the approved protocol.

6. A summary of the results as they relate to the study's objective.

7. The location where all raw data will be stored.

This report shall conform to all requirements outlined in Section 58.185, Subpart J, Good Laboratory Practices Regulations.

PROTOCOL C30B (Cont'd)

Test for Chemical Induction of
Unscheduled DNA Synthesis in
Primary Cultures of Rat Hepatocytes
(By Autoradiography)

Issue Date: December 14, 1982

Sponsor: James E. Weaver James E Weaver
Divisional Toxicologist

Date Approved by Sponsor's Divisional Toxicologist 3-7-85

Proposed Starting Date: May 1, 1985
Defined as Preliminary Cytotoxicity

Proposed Completion Date: June 19, 1985
Defined as Final Report Completion

To be completed by the Test Facility

Study Director: Rodgun D. Cunn

Date: April 16, 1985

Study Cost: $5,200.00

References:

1. G. M. Williams. The detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. Cancer Res. 37: 1845-1851 (1977).

2. G. M. Williams, E. Bermudez, and D. Scaramuzzino. Rat hepatocyte primary cell cultures. III. Improved dissociation and attachment techniques and the enhancement of survival by culture medium. In Vitro 13: 809-817 (1977).

DIVISIONAL REQUEST DOCUMENT (DRD)

DRD # BYCR0394

A Staff Originator

Originator: (Type/Print) T. J. Rollosson (Signature/Date) T J Rollosson 2/28/85

Business Dept. Managerial Concurrence (Signature/Date)

Originator's SH: Y. E. Ohling Originator's AD: [signature]

Charge # 4185-5128-7511 * Estimated Total Cost $ 5.5M

B Compounds, Formulas, and Products to be Tested

	TSIN #
Octopirox	60539.01

Material or Product Classification : Antidandruff active

C Safety Test Requirements

Test	Approx. Cost	Procedure	Amount of Substance Needed	Additional Comments	Test Facility**
Unscheduled DNA & Synthesis	5.5M	C30B	10g		Microbiol. Assoc.

D Risk Level Assignment for Human Safety Studies*

I ___
II ___ Has SRC approved study? NA/YES/NO
III ___ Has IRB approved study? NA/YES/NO

Name of Investigator (if in-house):

E Assignment of Risk and/or Agreement to Test Request

James E Weaver (Toxicologist's Signature) ___ (Name) 3/7/85 (Date)

Substances required by: ___ (Date) Accepted by Process: ___ (Signature/Date)

Concurrence with test request: (provide TSCR's before seeking this concurrence)

R H Barman Safety Section Manager 3/11/85 Date

[signature] P&RS Associate Director 3/14/85 Date

* To be completed by toxicologist
** To be completed by MVL Operations Liaison

For MVL Use Only:
Corporate Liaison [signature] 3/28/85 (Signature/Date)
Operations Liaison H. A. Dunn 3/25/85 (Signature/Date)

Issue Date 9/82.

BEAUTY CARE DIVISION

TEST SUBSTANCE CHARACTERIZATION REPORT*
(TSCR)

For Tox Office Use Only:
DRD # BX80394
TSIN # 60539.01

1. **Test Article Name (should agree with DRD):** Octopirox

2. **Making Notebook Ref. (with Plant Production Code if applicable):** Packing Ref: QC-0104-136

3. **Date Made:** Packed 2/25/85 **Made by:** N/A **Lab:** 1B20

4. **Physical Form:** White Powder **Color:** White **Density:** N/A

5. **Water Solubility:** <1 % (wt/wt)
   For SENS test only - **Ethanol Solubility:** >10 % (wt/wt)
   Acetone Solubility: >10 % (wt/wt)

6. **Sample Expiration Date:** 12/7/85 **Stability Testing is:** [ ] Completed [X] In Progress

7. **Hazards:** Irritant **Storage Conditions:** Ambient (50-90°F)

8. **D.O.T. Classification (see Regulatory Personnel):** Non-Combustible, Not Hazardous

9. **Microbial Susceptibility Classification Approval:** David J Kaufman (Signature)
   _____ (Name) 3/4/85 (Date)

10. **Making Data:** List ingredients in descending order of predominance.
    (For drug products list active ingredient(s) first.)

Component	Nominal Level (Theoretical % by wt.)	Actual Level (% by wt.)	Stock Code	Lot Number
Octopirox	100	100	BX288	1

Comments on Making Data: Packed into Pint Freezettes

*Submit typed original with DRD. Include copy of making records.

Issue Date 5/84

For Tox Office Use Only:
DRD #BYC02394
TSIN #G2539.01

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

11. Characterization, Microbial and Properties Information:

	Date Submitted	Submitter Code (if exists) or Lab Notebook #	Component or Property	(√)	Measured Value	Limits	Testing Lab or Data Source
1	2/27/85	JDM 0108	MCT	√	Pass	Must Pass	Micro
2	11/8/84	HC-0173-46	% Octopirox		100.26%	98% Min.	1B21
3	12/05/84	84312004	Assay		99.4	97% Min.	Analytical
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

12. Approvals:

The test substance as made and characterized is a representative example of the intended formulation. Making records for plant-made product should be obtained and evaluated by Products Research.

a. Process Development: J D Nelson (Signature) — John Nelson (Name) — 3/4/85 (Date)

b. Products Research: T J Nelson (Signature) — (Name) — 2/27/85 (Date)

_____ finished product samples will be retained by Quality Assurance.
samples

c. GMP-Quality Assur.: T Johnstone (Signature) — M Johnstone (Name) — 3/4/85 (Date)

---

13. The characterization tests requested are appropriate and the test substance is acceptable for: [X] acute animal test; [X] subchronic animal test; [X] chronic animal test; [X] human safety test; [ ] in vitro test; [ ] environmental safety test.

James E Woodson (Toxicologist's Signature) — (Name) — 3/26/85 (Date)

SCR Distribution: Original - Tox Office; Copies - Toxicologist, GMP/QA, Products REsearch and Process Dev.

057/M
bg

TEST ARTICLE CHARACTERIZATION

To comply with Good Laboratory Practice Regulations and to assist us in the proper handling and accurate evaluation of your test article, please provide the following information in English.

XX Test Article Identification: (TSIN) G0539.01

Lot Number: included in TSIN

Quantity Sent: included on label

Physical Description: included in protocol
(Color, state, viscosity, etc.)

Storage Requirements: included in protocol

Expiration Date: included on label
(Mo./Day/Yr.)

Purity: see protocol (if applicable)

Strength: see protocol (if applicable)

Stability: This is the responsibility of the sponsor.

Solubility: see protocol

Volatility: see protocol (if applicable)

LD_{50}: This material is being evaluated for safety.
(Specify species and route.)

XX Precautions in Handling or Disposal: IRRITANT - USE NORMAL PRECAUTIONS

In order to comply with RCRA, Department of Transportation and Maryland State regulations for the proper management of hazardous waste, we must have the following information before we can accept material for testing. If you wish to maintain secrecy of the test article identity during testing, this information can be provided to our Regulatory Affairs/Quality Assurance Department, which will assume the responsibility for managing the material and will not disclose the identity to laboratory personnel.

XX Chemical Class: ANTIMICROBIAL COMPOUND
e.g. shampoo, alcohol, etc.

XX Proper DOT Shipping Name: (if known)

XX DOT Hazard Class: (if known) NON-COMBUSTIBLE, NONHAZARDOUS

XX UN or NA Number: (if known)

EPA Waste Number: (if known)

XX Procter & Gamble Co.
Sponsor

H. A. Dunn for J. E. WEAVER
Signature of Authorized Representative

MVL P.O. Box 39175
Street Address/P.O. Box

4/11/85
Date

Cincinnati Ohio 45247
City State Zip Code

Form No. MA-261
1/20/84

MICROBIOLOGICAL ASSOCIATES

RTL
BYCR 0394

THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

August 1, 1985

Dr. Rodger D. Curren
Microbiological Associates
5221 River Road
Bethesda, MD 20016

Dear Dr. Curren:

In reviewing the following final report:

Report No.: T2982.380
Test Compound: G0539.01 (BYCR 0394)
Type Study: UDS

I noted the following:

The code for the test article on page 5, paragraph 3, is incorrectly shown as G0569.01.

Please send me three copies of the corrected page stating that it is a corrected page, signed and dated.

I would appreciate a response within two weeks, if possible.

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research and Development Department

H. A. Derner
Human & Environmental Safety Division

slh

cc: Study File
J. E. Weaver
N. Karten (MA)